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Brief Report

Preventive effects of hyaluronan from deterioration of gait parameters in surgically induced mice osteoarthritic knee model

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SUMMARY

Objective: Osteoarthritis (OA) leads to pain and loss of function in affected joints. Gait disturbance results from these symptoms of OA, and gait analysis can be important to evaluate the progression of OA. The purpose of this study was to analyze gait pattern in a rodent model of OA and to assess the effects of intra-articular injection of hyaluronan (IAI-HA) by gait analysis, along with histological evaluation.
Design: OA was induced by destabilization of the medial meniscus (DMM) of C57BL/6 mice. IAI-HA started 3 weeks after DMM surgery. Mice were allocated to three groups and were given either 800-kDa HA (800-HA), 6000-kDa HA (6000-HA) or saline. We compared these three groups with a sham group by gait analysis using CatWalk™. Histological evaluation was performed to assess articular cartilage changes in the knee joints.
Results: Mice injected with 800-HA or 6000-HA showed gait patterns similar to that of the sham mice, while the saline-injected group showed gait disturbances 12 and 16 weeks after DMM surgery. Histological changes in articular cartilage were similar among the 800-HA, 6000-HA and saline-treated groups, demonstrating OA progression throughout the experimental time points. Positive gait-related effects of IAI-HA might occur by its pain relieving effect and/or by preventing contracture.
Conclusion: IAI-HA prevented gait disturbances in the DMM model, but did not prevent histological changes associated with OA progression.

Introduction

Osteoarthritis (OA) leads to pain and loss of function in affected joints and causes limitations in patients' activity and diminishes quality of life[1]. Pharmacological interventions are still insufficient to modify the progression of OA[2].

To examine the efficacy of interventions, a variety of animal models have been developed. They are useful in histological evaluation but also allow us to study the behavioral changes. For that purpose, several behavioral assessments have been performed and reviewed in previous literature[3]. Among them, gait analysis on animal models may provide insights into the underlying OA progression and effects of interventions because gait is one of the important activities of daily living impaired by OA[4].

Recently, CatWalk™ (Noldus Information Technology, Wageningen, The Netherlands) has been reported as a useful device for gait analysis of rodent models[5]. But comprehensive gait analysis against the widely used surgically induced OA model, destabilization of the medial meniscus (DMM) model, has not been fully performed yet.

Intra-articular injection of hyaluronan (IAI-HA) is widely used and has clinically proven pain relieving effects and functional improvements[2]. In this study, using the DMM model, the efficacies of two different molecular-weight HAs were assessed by CatWalk™ along with histology of the knee joint.

We hypothesized that gait parameters would change as OA progresses, and that IAI-HA will have an effect on these changes in gait parameters in the DMM model.

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Materials and methods

Animals

C57BL/6 mice (9 weeks old, male, $n = 60$) were used. All protocols for animal procedures were approved by the Ethics Committee of Chiba University after the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (1996 version).

Induction of OA

Mice were anesthetized by intraperitoneal injection of ketamine (0.1 mg/g) and xylazine (0.01 mg/g). DMM surgery was performed as previously described[6]. In this model, transection of meniscotibial ligament leads to instability of the medial meniscus, which resembles the slowly progressing OA.

Experimental design

DMM was performed on the left knee joints of 45 mice. Fifteen mice underwent sham surgery on the left knee joint where only a skin incision and medial capsulotomy with luxation of the patella were performed. IAI was started 3 weeks after the DMM. The 45 mice with DMM were allocated equally to three groups, and were given IAI into their left knees: (1) single weekly 800-HA (800-kDa, Artz®, Seikagaku Corp., Tokyo, Japan) injection for 5 weeks, (2) single weekly 6000-HA (6000-kDa, Synvisc®, Sanofi Biosurgery, Cambridge, MA) injection for 3 weeks, or (3) single weekly saline injection for 5 weeks.

Gait analysis using CatWalk™ was performed before the surgery to obtain baseline data, and 3, 8, 12, 16 weeks after the surgery. After the gait analysis, mice ($n = 5$) were sacrificed at 8, 12, and 16 weeks from each group. Left knees were dissected and processed for histological evaluation.

IAI

For IAI, mice were anesthetized by the same method as DMM surgery and a small skin incision was made to identify the patellar tendon. With a 30G needle, injection was performed through the patellar tendon to ensure that HA or saline was injected into the joint securely. Also, a handmade styrene foam stopper was prepared and secured around the needle, so that the needle could only be inserted 3 mm, avoiding damage to the articular cartilage. The dose of each injection was 20 μl of 1% HA or 0.9% saline.

Gait analysis by CatWalk™

In CatWalk™ (Noldus Information Technology, Wageningen, Netherlands) system, mice are placed on a glass plate walkway and allowed to walk freely. Fluorescent light is emitted inside the glass plate and completely internally reflected. When the paws contact the glass plate, light is reflected downward and recognized as a bright image of the paw prints. These paw prints are captured by the video camera set beneath the walkway and were analyzed by software (CatWalk™ XT version 9) that generated the static gait parameters such as contact area and paw pressure, and the dynamic gait parameters related to duration of the step. The software automatically presents a total of 21 gait parameters.

Ten walks for each mouse were recorded. Three uninterrupted walks were selected out of 10 recorded walks. Selection was done by two observers (YM and YA) blinded to treatment. Gait analysis was conducted on five mice for each group, thus a total of 15 walks for each group were selected and analyzed at each time point. To consider individual differences between mice, the ratios of the affected hind limb to the contralateral hind limb (left/right ratio) of all 21 gait parameters were evaluated, and compared among groups. Mean ± 95% confidence interval of the ratios were expressed.

Histological evaluation

After the gait analysis, all mice ($n = 5$ for each group at each time point) were deeply anesthetized with intraperitoneal injection of ketamine (0.2 mg/g) and xylazine (0.02 mg/g), then perfused with 4% paraformaldehyde. The knee joints were dissected free of muscle and decalcified in 20% ethylenediaminetetraacetic acid for 21 days, then knee joints were embedded in paraffin blocks. Sections of 4 μm thickness were made in coronal plane, at approximately 50 μm intervals throughout the whole knee joint. Slides were stained by Safranin-O and Fast-Green.

The articular cartilage was assessed using the Osteoarthritis Research Society International (OARSI) histological scoring system[7]. Four quadrants of the joint; medial femoral condyle (MFC), lateral femoral condyle (LFC), medial tibial plateau (MTP), and lateral tibial plateau (LTP) were scored separately. For each quadrant, 10 slices that sample the entire joint were scored and each quadrant score was summed. The average scores of each group ($n = 5$) were compared among groups at each time point. Scoring was performed blindly by two authors (YM and HH). We also compared the scores of the lateral compartment (LTP, LFC) and the medial compartment (MTP, MFC) in each group at each time point, to see if OA progression or effect of intervention differs in two compartments.

Statistical analysis

Statistical analysis was performed using repeated measures analysis of variance (ANOVA) followed by Bonferroni corrections to compare longitudinal changes in each gait parameter within each group. Non-repeated measures ANOVA followed by Student–Newman–Keuls test was used to compare each gait parameter among groups. To compare histological scores among groups, Kruskal–Wallis tests followed by Steel–Dwass tests were used. Mann–Whitney's U tests were used to compare the histological scores of the medial and the lateral compartment. A P-value less than 0.05 was considered statistically significant.

Results

Gait analysis by CatWalk™

In the saline-treated group, no significant changes were seen in the left/right ratio of any gait parameter through 8 weeks, but significantly shorter stance phase, single stance phase, duty cycle (percentage of stance phase during the step cycle), and swing speed, and a significantly longer duration of swing phase were observed at 12 and 16 weeks compared with the baseline data (0 week). In contrast, the 800-HA and 6000-HA groups showed a gait pattern similar to that of the sham group, in which the ratio of gait parameters did not change during the entire period. Gait parameters that changed longitudinally in the saline-treated group were significantly different compared to the other groups at 12 and 16 weeks (Fig. 1). There were no statistical differences among sham, 800-HA or 6000-HA groups at any time period.

Histological evaluation

Histological changes were not apparent in the sham group, whereas the saline-treated group demonstrated OA progression